VOLUME-8, ISSUE-8, AUGUST-2019 • PRINT ISSN No. 2277 - 8160

Original Research Paper | Genetics

# ABSENCE OF AN ASIAN FOUNDER MUTATION C.100C > T(P.R34X) IN TMC1 AMONG A LARGE COHORT OF PRELINGUAL NON SYNDROMIC HEARING LOSS FROM SOUTH INDIA


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## ABSTRACT

**Aim:** To investigate the frequency of an Asian founder mutation c.100C>T (p.R34X) that dominates the TMC1 gene mutation spectrum, in a large cohort of prelingual hearing impaired (HI) from south India.

**Methods:** A total of 360 children from deaf schools and 116 prelingual adult HI from assortative mating cohort were included in the study, after excluding for the common GJB2 etiology. We determined the genotype of the probands using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method.

**Results and discussion:** p.R34X mutation was not found in all the 476 hearing impaired screened in our cohort unlike other studies who reported low prevalence of TMC1.

**Conclusion:** We conclude that c.100C>T mutation is not a significant cause of deafness in the south Indian population. However the actual frequency of TMC1 gene mutations contributing to deafness, can be obtained only by sequencing all the 24 exons of the gene.


**KEYWORDS :** Deafness, TMC1, p.R34X, founder mutation, south India

## INTRODUCTION

In south India the occurrence of deafness is fairly significant with an incidence that is six times the global average (Yan et.al., 2015). In our search for the causative genes for hereditary hearing loss in a unique cohort of assortative mating south Indian population, we found that 29.8% (49/164) is due to mutations in the common *GJB2* gene alone (Amritkumar et.al., 2018). After excluding for *GJB2*, we further accounted the frequency of mutations in other genes like *SLC26A4* [4.4% (5/113)] (Chandru J, unpublished thesis, 2017) and *CDH23* [3.4% (4/116)] (Vanniya et.al., 2017) in the same population. We also screened children from deaf schools in south India for *GJB2* gene mutations and accounted for about 19.8% (78/394) (Selvakumari M, unpublished thesis 2015).

Routine clinical testing options beyond *GJB2* in a cost effective manner is still a challenge in India. In order to further dissect the relative contribution of a less explored Asian specific founder mutation c.100C>T (p.R34X) in another auditory gene *TMC1* (cochlear-expressed, transmembrane channel-like gene 1), we continued the screening in these two cohorts for this mutation.

The *TMC1* gene underlies both recessive and dominant nonsyndromic hearing loss at the DFNB7/B11 and DFNA36 loci with variable clinical presentation (Kurima et.al., 2002). The p.R34X founder mutation results in a C-to-T transition (located in exon 7) at position 100 from the first ATG (c.100C>T), thus, creating a premature stop codon. It results in recessive deafness due to loss of function pathogenesis. This mutation was also observed in two previous Indian studies so far reported (Ganapathy et.al., 2014; Singh et.al., 2017). However, Kurima et.al., (2002) did not find this c.100C>T mutation among the two Indian families screened in a cohort

**Table 1: Worldwide prevalence of *TMC1* mutations among different populations till date (May 2019)**

| Population | Subjects | Mutations identified | Mutation type | Prevalence | Reference |
|---|---|---|---|---|---|
| North American | LMG128 family<br>230 consanguineous | DFNA36 1714G→A (D572N) c.100C→T (R34X) | Missense<br>Nonsense | 5.2%, (12/230)=2.2) | Kurima et.al.,2002 |

| Pakistan and India | ARNSHL families | c.1534C→T (R512X)<br>c.295delA<br>c.1960A→G (M654V)<br>del(IVS3_IVS5del27kb)<br>IVS13+1G→A<br>IVS10–8T→A | Missense<br>Deletion<br>Missense<br>Deletion<br>Splice site<br>Splice site | | |
|---|---|---|---|---|---|
| Pakistan | 168 highly consanguineous ARNSHL families negative for Cx26 | c.830A>G (p.Y277C),<br>c.1114G>A (p.V372M),<br>c.1334G>A (p.R445H),<br>c.2004T>G (p.S668R)<br>c.2035G>A (p.E679K),<br>c.536 -8T>A | Missense<br>Missense<br>Missense<br>Missense<br>Missense<br>Splice site | 4.8% (8/168) | Santos et.al., 2005 |
| Pakistan | 557 ARNSHL large Pakistani families | VS3_IVS5del27kb<br>IVS511G.T<br>c.100C  T (R34X)<br>IVS10-8T.A<br>VS1311G.A<br>c.1534C.T (p.R512X)<br>c.1541C.T (p.P514L)<br>c.1543T.C (p.C515R)<br>c.2004T.G (p.S668R) | Deletion<br>Splice donor site<br>Nonsense<br>Splice acceptor site<br>Splice donor site<br>Missense<br>Missense<br>Missense<br>Missense | 3.4% (19/557)<br>R34X =  1.8% (10/557) | Kitajiri et.al., 2007 |
| Pakistan | 6 families (WES) | p.R34X<br>p.R389Q<br>c.1788C>A (p.S596R)<br>c.596A>T (p.N199I)<br>c.1404+1G>T | Nonsense<br>Missense<br>Missense<br>Missense<br>Splice site | 5/6 | Imtiaz et.al., 2016 |
| Tunisian | 85 consanguineous and 60 unrelated cases | c.100C ] T (p.R34X),<br>c.1165C ] T (p.R389X)<br>c.1764G ] A (p.W588X) | Missense<br>Missense<br>Missense | 5% (7/145)<br>R34X = 3.4% (5/145) | Tlili, et.al., 2008 |
| Tunisian | 4 families (WES) | c.2260+2T>A | Spllice site | - | Riahi et.al., 2014 |
| Iran | 2 families (Linkage) | c.776+1G>A<br>c.1589_1590delCT;<br>p.S530* | | - | Hildebrand et.al., 2010 |
| Iran | 45 ARNSHL families | c.-258A>C | | 2.2% (1/45) | Davoudi-Dehaghani et.al., 2013 |
| Iran | 37 ARNSHL families | Linkage to TMC1 in 1 family | | 2.7% (1/37) | Tabatabaiefar et.al., 2011 |
| Iran | 144 ARNSHL families | IVS7+1G>A<br>c.1589–1590delCT<br>c.150delT | Splice site<br>Deletion<br>Deletion | 2.8% (4/144) | Babanejad et.al., 2012 |
| Population | Subjects | Mutations identified | Mutation type | Prevalence | Reference |
| British Asians | 476 unrelated deaf individuals | c.100C  T (R34X) | Nonsense | R34X=0.2% (1/476) | Searle et.al., 2012 |
| Sudanese | 243 unrelated deaf individuals | c.1165C>T (p.Arg389X) | Nonsense<br>Splice site | 1.6% (4/243) | Meyer et.al.,2005 |
| China | 1 family (WES) | c.1253T.A (p.M418K) | Missense | | Zhao et.al., 2014 |
| China | 4 families (High throughput sequencing) | c.1253T>A (p.M418K)<br>(c.1714G>A) (p.D572N)<br>(c.[797T>C]) (p.[I266T]<br>c.2276G>A (p.R759H) | Missense<br>Missense<br>Missense<br>Missense | | Wang et.al., 2018 |
| Moroccan | 1 family WES | c.1810C > G (p.Arg604Gly) | Missense | | Bakhchane et.al., 2015 |
| Polish | 2 families WES | p.S320R | Missense | | Hassan et.al., 2015 |
| Belgium (Europe) | 24 ARNSHL individuals (Targeted sequencing) | c.458G>A (p.W153X)<br>c. 763þ3A>G<br>compound heterozygous | Nonsense<br>Splice site | (1/24) | Schrauwen et.al., 2013 |
| Turkey | 65 ARNSHL families negative for Cx26 | c.776A>G [p.Tyr259Cys],<br>c.821C>T [p.Pro274Leu],<br>c.1334G>A [p.Arg445His],<br>c.1083_1087delCAGAT [p.Arg362ProfrX6]. | Missense<br>Missense<br>Missense<br>Deletion | 6% (4/65) | Kalay et.al., 2005 |
| Turkey | 86 ARNSHL families negative for Cx26 | p.G444R (c.1330G>A),<br>p.R445C (c.1333C>T),<br>p.I677T (c.2030T>C),<br>IVS6+2 T>A<br>(c.64+2T>A),<br>Deletion of exons 19–24<br>p.R34X (c.100C>T). | Missense<br>Missense<br>Missense<br>Splice site<br>Deletion<br>Nonsense | 8.1% (7/86)<br>R34X=1.2% (1/86) | Sirmaci et.al., 2009 |

| Turkey | 49 NSHL families negative for Cx26, two Cx30 genomic deletions and a mitochondrial mutation in MTRNR1, 1555A.G | c.582G>A<br>31kb deletion<br>c.1333C>T<br>c.64+2T>A | | 6.6% (4/49) | Duman et.al., 2011 |
|---|---|---|---|---|---|
| Turkey | 51 familial patients with ARNSHL | c.100C>T (p.R34X)<br>c.1165C>T (p.R389X)<br>c.2350C>T<br>c.776+1G>A<br>c.767_768del<br>c.1166G>A | | 1.4% (7/51)<br>R34X=<br>3.9%(2/51) | Hilgert et.al., 2008 |
| India | 374 ARNSHL families negative for Cx26 | c.100C.T (p.R34X)<br>c.237-6T.G<br>c.453+2T.C<br>c.628_630del (p. I210del)<br>c.800G.A (p. G267E)<br>c.1114G.A (p. V372M)<br>c.1333C.T (p. R445C)<br>c.1566+1G.A | Nonsense<br>Splice site<br>Splice site<br>Deletion<br>Missense<br>Missense<br>Missense<br>Splice site | 1.6% (6/374)<br>R34X=(1/374) | Ganapathy et.al., 2014 |
| India (North) | 41 famiilies NSHL<br>50 controls | c.1283C>A (p.Ala428Asp)<br>c.100C>T (p.R34X) | | 4.9% (2/41)<br>R34X=(1/41) | Singh et.al., 2017 |

comprising of both Indians and Pakistanis. Till date, 59 different types of DFNB7/11 variants and four DFNA36 variants have been identified worldwide (Wang et.al., 2018) (Table 1).

## MATERIALS AND METHODS

After excluding for *GJB2* etiology, a total of 360 children from deaf schools and 116 prelingual HI from assortative mating families [deaf marrying deaf (DxD) and deaf marrying normal (DxN)], were screened for the c.100C>T (p.R34X) mutation in *TMC1*. This study was approved by the institutional ethical review board; written informed consent was obtained from all the probands.

Genotyping of p.R34X mutation was done through PCR-RFLP by means of a previously described allele specific restriction digest using *Taq1* restriction enzyme (Tlili et.al., 2008). Standard PCR amplification of *TMC1* exon 7 was carried out in a total of 476 probands, using the following primers: forward, 5'-AAG CAC TTT CTG ACCA TTA CTC ATT G-3'and reverse, 5'-TGG AAC TTT TGA AAG AAT ATC AGA-3'. The 250bp amplicon was cut with *Taq1* and resolved on a 3% agarose gel. Absence of p.R34X results in two bands of 171 and 79bp, while when the mutation is present, the 250bp band persists.

## RESULTS AND DISCUSSION:

On genotypic analysis, we found that none of the 476 south Indian HI probands harbored the Asian founder mutation p.R34X in *TMC1* gene (Figure 1).

The *TMC1* mutation spectrum is marked by a founder mutation c.100C>T (p.R34X) that is reported frequently among North African (Tunisia) and Asian (Pakistan) populations and accounts for 30% of all *TMC1* mutations (Ben et.al., 2010). Mutations in the *TMC1* gene have been reported as a common cause for deafness in India and Pakistan accounting for 5.4%(Kurima et.al., 2002) of which p.R34X alone accounts for 1.8% in Pakistani families.

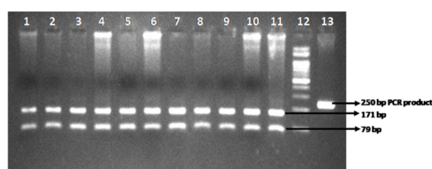


**Figure 1:** RFLP analysis of p.R34X mutation using *Taq1* restriction enzyme. Lanes1-11 show two bands of 171bp and 79bp representing the presence of wildtype alleles. Lane 12 shows the 100bp DNA marker and Lane 13 represents the uncut PCR product.

In Indian subcontinent combining the three previous *TMC1* gene mutational frequencies (Kurima et.al., 2002; Ganapathy et.al., 2014; Singh et.al., 2017), an overall frequency of 3.26% (14/423) is estimated, of which p.R34X mutation accounts to 0.5% (2/421).Taking advantage of this finding, we substituted the whole gene screening of *TMC1* with a rapid and cost effective screening of an Asian founder mutation in our geographically well-defined south Indian population. Although two previous Indian studies have reported the presence of p.R34X mutation (Ganapathy et.al., 2014; Singhet.al., 2017), we did not find this site as a hot spot in our south Indian cohort that is essentially Dravidian in origin.

South Asian ethnic groups consists of populations hailing majorly from eight countries that include Pakistan and India. In terms of linguistic groups these two countries fall into either Indo-Aryan or Dravidian societies. The distribution of p.R34X mutation is varied among South Asians. This may be due to the differences in the linguistic origin. The report by Ganapathy et.al., (2014) was a multicentric study and the cohort consisted of a mixed population hailing from both north and south India, while in Singh et.al., (2017) study the cohort hails exclusively from north India. This mutation was reported in a single case each, that must have originated from the northern region of India whose ethno-linguistic affiliation is Indo-Aryan.

A study by Searle et.al., (2012) reported a low frequency of 0.21% (1/476) and concluded that the common *TMC1* mutation c.100C > T (p. R34X) is not a significant cause of Deafness in British Asians, although this cohort was over represented by Mirpuri/Kashmiri of Indo-Aryan origin.

## CONCLUSION:

Thus, our study concludes that the Asian founder mutation c.100C > T (p.R34X) in *TMC1* gene, is not common among the south Indian HI population. Further, there is a need to carry out mutation screening in the whole gene to establish the absence of other mutations in *TMC1* as a major contributor to deafness in south India.

### Acknowledgements

We are grateful to the deaf families for their cooperation and participation in this study. We appreciate the support of Dr. Pavithra Amritkumar for sharing the *GJB2* mutational profile of the assortative mating cohort. We are thankful for the financial assistance provided by Ad hoc Research Project (5/8/10–17(Oto)/ CFP/2011-NCD-I) of the Indian Council of

Medical Research (ICMR), during the first phase and currently by DBT multicentric grant (BT/PR26850/MED/12/808/2017 dated 03.01.2019) to CRS. During the execution of the project, GN and JJM were supported by CSIR-SRF, PVS by ICMR-SRF and JC and SN by UGC-BSR fellowship.

**Author Disclosure Statement**

No Competing financial interest exist.

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